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THE

AMERICAN NATURALIST

VOL. XXXIV. *July, 1900.* NO. 403.

NOTES ON A SPECIES OF PELOMYXA.

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IN rearing amœbas for class use, as every one knows, many interesting protozoa make their appearance. The Pelomyxa described in this communication developed in great numbers, something over a year ago, in a "culture" which had been prepared for this purpose. The method of preparation was one that I have employed for several years, and is as follows. Into an ordinary wooden tub (2 ft. by 1 ft.) sand is poured to a depth of four inches. The tub is then put under the tap of a table aquarium, and flushed until the water is comparatively clear. A good handful of Nitella, two or three opened mussels, and a crayfish, cut into a couple of pieces, are thrown into the water and partially imbedded in the sand. The tub is left exposed to a north light from large windows, at a distance of some yards from the windows. As decomposition progresses, a very gentle stream of water is turned on for a short time every few days. After an interval varying from two or three weeks to two months, large amœbas (*A. proteus*) are found in abundance in the surface layer of the sand, and on the sides of the tub. Small amœbas frequently appear in numbers before the large ones come on. The commonest small form to appear is *A. radiosa* — occasionally *A. limax* (or some similar form). I have tried these tub cultures only during the warmer months of the year (April 1 to November 1). With rare exceptions they are successful. Mussels alone have frequently given me good amœbas, but the Nitella and the crayfish increase the chance of having enough to start with. The sand may come from anywhere, roadside or creek. The water is well water pumped into a reservoir tank. I am inclined to believe that a trace of mud, got along with the mussels or Nitella, is an advantage. But anything more than a trace is, here, sure to result in the development of Tubifex, which quickly spreads over the bottom, strewing it with fæces, while the amœbas, even if they get a start, gradually become scarcer, soon disappearing.

In the succession of organisms developing in such a culture, the bacteria are followed by the flagellate, and then by the ciliate Infusoria, both especially abundant in the surface film, where they feed upon the zoöglœa. A loose brown growth accumulates on the bottom, but more thickly on the wall of the tub. This consists of many things: fungus, hyphæ, bacteria, unicellular algæ, quantities of Infusoria, often Heliozoa (Actinophrys), and rotifers. The stuff gradually falls from the wall and accumulates on the bottom, round the edge. In this "growth" the small amœbas referred to above are often found, and in it *Stentor cœruleus* frequently develops in the greatest abundance. It is usually after this growth has begun to accumulate on the bottom, round the edge, that the large amœbas make their appearance. They are more abundant over the general surface of the sand than in the thick masses of brown stuff. By this time the Infusoria are no longer present in excessive numbers. The Entomostraca soon develop, and the amœbas gradually disappear. I think Cyclops is particularly destructive — it is constantly "rooting" in the sand. Frequently, before the arrival of the Crustacea, small planarians appear in numbers — *Microstomum lineare* has swarmed several times.

In a culture of this kind (mussels and Nitella both taken from a creek, the bottom of which in the particular locality is made up of soft mud, with abundant Tubifex), that had been going during August, there appeared in the early part of September numbers of a large rhizopod belonging to Greef's genus Pelomyxa. After discovering the presence of the form, I examined the tub systematically and found that the Pelomyxas were about evenly distributed over the wall, and in the stuff round the edge, but were not present over the bottom, where Tubifex had already made its appearance. They were abundant. On the average, each pipetteful of stuff (about 2 cc.) contained a specimen, which could be detected at once with the naked eye in a watch glass. In the same brown growth *Stentor cœruleus* was present in great numbers, and the Pelomyxas were feeding chiefly on these. In the Pelomyxas recognizable pieces of Stentor were commonly present, and sometimes a whole Stentor still rotating. Unlike the Pelomyxas previously described, this form contained no mud nor sand.

The Pelomyxas continued to be abundant in the tub (preserving the same distribution) for nearly two months. Towards the end of this time they began to disappear, the Stentors going first. With the gradual disappearance of these forms, Crustacea, particularly Cyclops, developed. The probable share that Cyclops had in the destruction of the Pelomyxas was indicated clearly in the following case. On September 8, ten Pelomyxas were transferred to a small glass aquarium jar, together with some of the brown "growth" and a little Spirogyra. They were supplied, during the first few days, three times with Stentors in considerable number. Within five days the Pelomyxas increased in number to fifty odd. After a week or two, Cyclops began to appear, and the Pelomyxas diminished in number and soon disappeared. I never actually saw Cyclops seize a Pelomyxa, but the former was constantly darting into, or "rooting" in, the stuff in which the rhizopods lay. And the only animals observed in the jar, besides Cyclops and Pelomyxa, were small Infusoria.

In the following case the Pelomyxas obviously disappeared because of gradual starvation. In this case a number of the rhizopods were transferred, in September, to glass preparation dishes, of a size (30 mm. × 70 mm. in diameter) that would permit examination under a low objective. In these dishes was kept clear water without any of the brown stuff or sediment, and the Pelomyxas were changed to fresh water occasionally. No Cyclops or other Crustacea developed in these dishes. For a time *Stentor cœruleus* was supplied, and later a small brown Stentor, Paramœcium, and other Infusoria. The Pelomyxas remained active and kept their size during this period, though they did not materially alter in number. After a few days they were no longer fed, though the dishes were kept supplied with fresh water. They gradually decreased in number, diminished very noticeably in size, and were very sluggish in their movements. The last of these individuals were made use of in early November. These starved individuals were about one-third the size of those originally put in the dish. It is possible of course that some division went on during this period of starvation, and that the shrinkage in size was not due merely to the loss of tissue.

To this form I have given the name of *P. carolinensis.* Its points of difference from the related species of Pelomyxa will be discussed later.

Description of Pelomyxa carolinensis.

Habitus. — The body, when viewed by reflected light, is white and conspicuous. When left for some time undisturbed (in small aquarium dishes suitable for microscopic observations), the body assumes a great variety of shapes, many of them indicating a high degree of consistency in the superficial layer of protoplasm. Owing to the large size of the species, these shapes may, with some success, be made out even with the unaided eye, though better with the help of a simple lens and low objective.

The body is very apt to assume an elongated, more or less rod-like shape, as in Fig. 1; sometimes enlarged at one end (clavate), as in Fig. 2. The elongated body may lie flat on the supporting surface, as in Figs. 1 and 2. More frequently the body is thrown into one or more curves, the animal resting on the bottom by its two ends, as in Figs. 3 and 4. Occasionally a Pelomyxa was observed in the remarkable attitude shown in

Fig. 5, a portion of the body resting on the bottom, while the rest of the animal, divided at the end into pseudopodia, projected freely upwards in the water. In this condition, Pelomyxa appears for the time being as an attached rhizopod with tentacle-like pseudopods, the nearest analogue to which is the interesting minute form *Stylamœba sessilis*, found by Frenzel[1] at Cordoba in Argentina.

If the animal in the undisturbed condition is not more or less rod-like, the body is frequently divided up into very long, slender pseudopodia, as in Figs. 6 and 7. In this condition the animal rests on the tips of certain pseudopodia, the rest of the body not touching the surface. The other pseudopodia project freely in the water in various directions, and the whole body is thrown into boldly arching curves, which usually lie in several planes. When the animal is in this condition, with its irregular body extending in so many directions, it obviously dominates (*i.e.*, can gather food from) a much larger cubic space than when in the simple rod-like condition. Now in that aquarium jar in which the Pelomyxas were best fed, and in which they increased so considerably in number (ten to fifty in five days), some form of the rod-like shape was nearly universal. Here the food (Stentors and other Infusoria) was most abun-

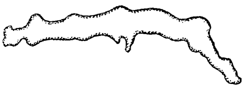

FIG. 1.

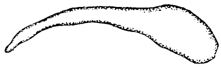

FIG. 2.

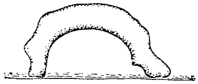

FIG. 3.

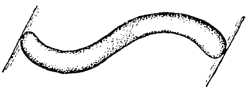

FIG. 4.

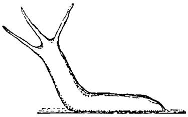

FIG. 5.

FIG. 1.— *P. car.* as an opaque object. Simple, elongated condition. Zeiss $a_2 \times 4$. Reduced to ½.

FIG. 2.— *P. car.* as an opaque object. Clavate condition. Zeiss $a_2 \times 4$. Reduced to ½.

FIG. 3.— *P. car.* as an opaque object. Body an arc resting on the two ends. Zeiss $a_2 \times 4$. Reduced to ½.

FIG. 4.— *P. car.* as an opaque object. Body somewhat spirally curved, resting on its ends. Zeiss $a_2 \times 4$. Reduced to ½.

FIG. 5.— *P. car.* as an opaque object. Body in part resting on bottom, in part protruding up into water, with tentacle-like pseudopods. Zeiss $a_2 \times 4$. Reduced to ½.

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[1] Untersuchungen über die mikroskopische Fauna Argentiniens. Protozoa. *Bibliotheca Zoologica*, Heft 12, 1892–97.

dant in the brown "growth," with which the bottom was strewn. With the food thus distributed, the worm-like shape was doubtless well adapted to the situation. On the other hand, in those dishes containing no sediment or brown "growth," but simply clear water, and in which the Pelomyxas did not increase in number, the irregular, branched condition (Figs. 6 and 7) was extremely common. In these dishes it is evident that food was scarce from the start, and the peculiar shape of the Pelomyxas is probably to be regarded as an adaptation to circumstances. We may think of the animals as thrusting their substance out in all directions of space, searching for food.

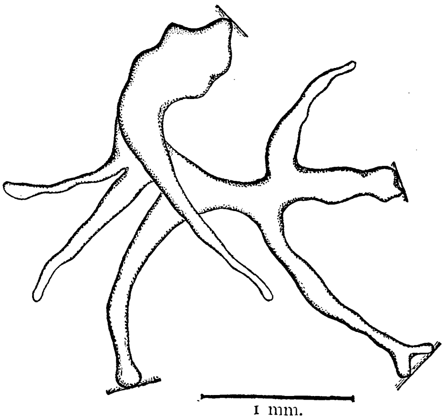

FIG. 6.

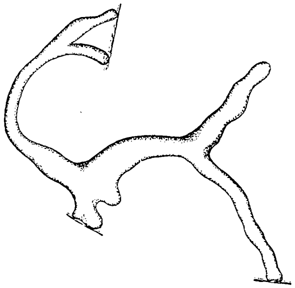

FIG. 7.

FIG. 6. — *P. car.* as an opaque object. Body thrown into bold curves and complexly branched, resting on bottom (indicated by lines) at four points. Remaining pseudopodia project freely into water. One millimetre drawn to same scale. Zeiss *A* × 2. Reduced to ½.

FIG. 7. — *P. car.* as an opaque object. Body thrown into two curves, lying in planes nearly at right angles with each other. Body rests on bottom (indicated by lines) at four points, one pseudopodium projecting freely upwards. Zeiss *A* × 2. Reduced to ½.

When brought on a slide in a drop of water, these animals assume the general shape of *Amœba proteus*. The body expands in a horizontal plane, and is, as a whole, applied to the surface over which it is creeping (Fig. 8). This is the case whether the drop be uncovered or covered — cover was always supported with wax feet, so as to permit free movement. This flattened shape is not directly caused by confinement between two surfaces (surface film and slide, or cover and slide), because it is also usually assumed when the Pelomyxa is first placed in a watch glass with plenty of water. It would appear to be the shape into which the amœboid body, more or less contracted after the reception of a shock, normally expands, before assuming any more characteristic habitus. If the Pelomyxa, owing to the shock of having been transferred to the watch glass, has contracted into a very compact shape, it may (rare condition) throw out short radial pseudopodia all over its body, excepting the lower surface (applied to the glass), Fig. 9. This condition is transitory, soon passing into the stage shown in Fig. 8.

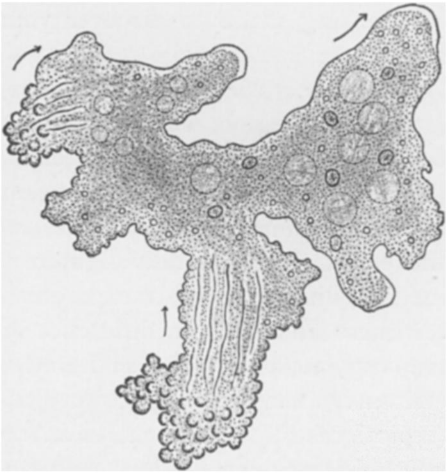

FIG. 8. — *P. car.* moving freely, on slide, under supported cover. Arrows indicate direction of endosarcal currents. Longitudinal, and papillose wrinkling at the ends of pseudopodia, out of which current is flowing. Only the most conspicuous vacuoles, nuclei, and albuminous globules represented. Zeiss *A* × 2. Reduced to ⅔.

Size. — In the relatively contracted shapes (Figs. 8 and 9) *P. carolinensis* measures about 1 mm. in diameter. The largest form actually measured, in which the body was of the slender irregular character shown in Figs. 6 and 7, measured in a straight line from pseudopodium tip to pseudopodium tip 2.8 mm. Some two hundred specimens of this species were examined by myself and the class demonstrator during September; and for the elongated shapes, the length may be said to vary from 1 to nearly 3 mm., the common length being 1.5 mm. to 2 mm. These measurements may

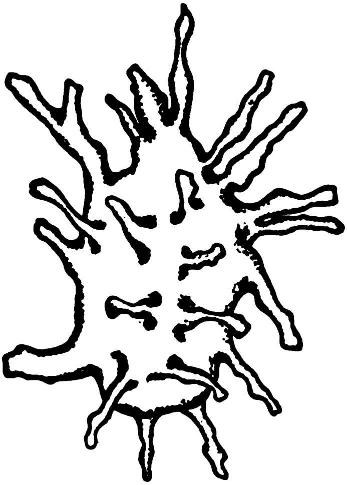

FIG. 9. — *P. car.* as an opaque object. Shortly after having been transferred to watch glass. Body covered with short radial pseudopods. Zeiss a_2 × 4. Reduced to ⅔.

be taken as characteristic of the adult condition, since the Pelomyxas were always picked out with the unaided eye, no effort being made to discover young forms with the microscope.

Structure of the Body. — There is a perfectly clear peripheral (ectosarcal) region, which in the living state is very narrow, being conspicuous only at the ends of expanding pseudopodia (Fig. 8). The granular, more opaque and fluid endosarc contains numerous nuclei, vacuoles, and other bodies. Though the endosarc is naturally lighter near the edge than further in, this difference in transparency is due to a difference in thickness and not to a difference in composition. The nuclei are very abundant, and scattered without order through the endosarc. They are elliptical in shape, and measure $18\,\mu \times 16\,\mu$ (typical case). The vacuoles in the endosarc vary greatly in size. Large ones, distinct with an a_2 (Zeiss) objective, and often measuring $40\,\mu$ in diameter, are scattered about in some abundance. When the body is compressed, or when sections are examined, the endosarc is seen to be honeycombed with vacuoles of all sizes; the very small ones, a few microns in diameter, being especially abundant. So abundant are the vacuoles in parts of the endosarc that the optical effect (in section or thinly expanded pseudopodium) is that of a spongy reticulum.

There is no contractile vacuole in this form. If a vacuole disappears, it does so simply because of the shifting of the protoplasm. The endosarc contains in the greatest abundance minute crystals, which contribute very materially to its dark appearance with transmitted light. These bodies are of an elongated fusiform shape, about $2\,\mu$ in length. When seen end on, they look like dots. They are readily soluble in alcohol and in dilute acetic (used in acetic carmine and methyl-green solution), but are neither stained nor dissolved by one per cent osmic. Their diminution in size and gradual disappearance, when treated with the above solvents, may be watched under the microscope. It requires but a few minutes for them to vanish completely, leaving the other inclusions much more distinct than they formerly were.

Scattered abundantly through the endosarc are spherical bodies, having in the living animal a bright appearance with a dark contour, and looking much like oil drops. They very commonly have a diameter of about 8 microns, though smaller ones of all sizes are present. These bodies evidently correspond to the "Glanzkörper" originally described by Greef[1] in *P. palustris*, although in the latter form they may reach a much larger size than I have observed in *P. carolinensis*. These "refringent bodies," as Gould[2] calls them, are insoluble in alcohol. They are stained by osmic acid and by iodine (alcoholic solution), in nowise differently from the granules of the endosarc (*i.e.*, are browned). They cannot, therefore, be of a fatty or starchy nature. The contents of the bodies is fluid, as may be demonstrated by allowing the Pelomyxas to be gradually compressed through the slow evaporation of water from under the cover-glass. At a time when the arrangement of the coarser and finer granules of the protoplasm is not interfered with, the globules burst and run together, suggesting fat droplets very strongly, but even in this condition osmic acid does not blacken them. On the other hand, the globules are stained a deep blue with hæmatoxylin. In sections (fixed in Zacharias, stained with Delafield's hæmatoxylin) it may be seen that the endosarc is thickly studded with coarse granules, which stain blue with hæmatoxylin. The smallest refringent bodies, recognizable as such, differ but little in appearance from these coarse granules. They are slightly larger, of a more rounded shape, and take a deeper stain. From these minute globules, all gradations in size may be found up to the large ones, 8 microns in diameter. With the increase in size the depth of coloration increases — an effect due doubtless to the greater diameter. The bodies, when stained with hæmatoxylin, present a perfectly homogeneous appearance. From the evidence at hand, it would seem to me that the bodies are globules of an albuminous nature, consisting of a pellicle, enclosing a more fluid substance. Greef (*loc. cit.*) inclined to the belief that the "Glanzkörper" were of intranuclear origin, and were concerned in reproduction. Gould (*loc. cit.*), working on *P. palustris*, failed to see evidence for the truth of these ideas, and in the form which I have studied there was no such evidence. Gould found the "Glanzkörper" to have a homogeneous appearance when stained with ordinary stains (the exception does not seem to have any special significance); and she reached the conclusion that they were "almost certainly either solid structures or filled with coagulable fluid." With the latter alternative I agree.

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[1] *Arch. f. mikr. Anat.*, Bd. x, 1874.

[2] Notes on the Minute Structure of Pelomyxa palustris, *Quart. Journ. Micr. Sci.*, vol. xxxvi, 1894.

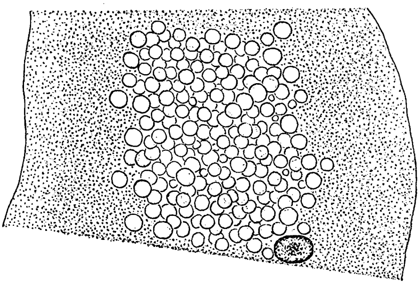

FIG. 10. — Portion of a starved Pelomyxa. Crystals dissolved out with alcohol. Drawn in water. Zeiss $D \times 4$. Reduced to ⅔.

The condition of the albuminous globules in starved individuals deserves a word. In healthy, active specimens the globules are scattered all through the endosarc. In certain individuals kept without food in pure water for several weeks, the globules were much more numerous than in normal specimens, and were aggregated together in several regions of the body. In each such region (Fig. 10) the globules were thickly crowded, while elsewhere they were nearly absent. These starved Pelomyxas were about one-third the common size of healthy specimens, and were exceedingly sluggish in movement.

In specimens of Pelomyxa kept without food for some time the body contains no inclusions except such (granules, crystals, albuminous globules) as seem to be normal and constant constituents. Owing to its large size and freedom from foreign inclusions, *P. carolinensis* presents certain marked advantages for the study of the fundamental structure of protoplasm. As a living object it is perhaps no better than smaller rhizopods, though under a cover-glass the pseudopodia may flatten out into very thin sheets. On the other hand, it is easy to handle for sections, and in sections the vacuolar walls in particular are instructive. One of the large vacuoles may be cut into several sections, and will thus afford both true sectional and surface views of its wall. For comparison with the living animal I have found it useful to employ surface preparations of specimens mounted in glycerine, after killing with acetic carmine or acetic methyl green; or surface preparations of specimens killed in alcohol and mounted in water. Acetic carmine (45 per cent acetic) kills the animal as quickly as an osmic fixative, the outline of the living state being faithfully retained. Alcohol and acetic methyl green kill more slowly, the animal undergoing some change of shape, which involves especially the peripheral region. On addition of these fluids the coarsely granular endosarc either contracts, or the surface film of the Pelomyxa is raised up by absorption of fluid. That the latter is the case, is the impression made on one when the animal is kept under observation during the action of the fixative — the surface seems to rise up often in bleb-like swellings. The result, at any rate, is that the clear ectosarcal region, very narrow in life, is greatly increased in width, and thus a considerable area, exhibiting the fine reticular (alveolar) structure characteristic of this region, may be had for study.

The general endosarc lying between the vacuoles shows the greatest abundance of coarse granules so closely set that they must obscure whatever finer structures are here present. These granules very commonly have a diameter of one micron, though smaller ones of varying size are abundant.

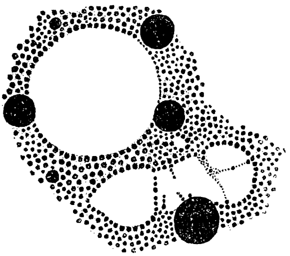

FIG. 11. — Part of section showing vacuoles, deeply stained endosarcal granules, and several deeply stained albuminous globules. Zeiss $\frac{1}{12}$ × 4. Reduced to $\frac{2}{3}$.

In sectional view (Fig. 11) the walls of many of the larger vacuoles seem made up of similar coarse granules, arranged regularly so as to form a smooth bounding surface for the cavity. These bounding granules are generally about 1 μ in diameter, but in places they may be much smaller, ranging down to the size of microsomes. Long vacuoles are occasionally found somewhat constricted, so as to be incompletely divided up into chambers (crenellated vacuoles of Gould, *loc. cit.*), as in Fig. 11. Intravacuolar strands may be seen in these vacuoles. In some cases the strands are undoubtedly the free edges of folds. Some of the strands consist of a single row of comparatively large granules (intermediate in size between the microsomes and the coarse endosarcal granules), easily counted, and which are connected by the finest lines (fibres), in which no structure can be made out with $\frac{1}{12}$ Zeiss. Other intravacuolar strands are of considerable thickness, appearing as a mass of finest granules (microsomes) closely set, in which a coarse granule is found here and there. Still other strands appear as exceedingly delicate lines, made up of one row of microsomes.

In surface views of vacuolar walls, studied in sections, the same appearance is not always had. In many cases the wall seems made up of microsomes, arranged so as to give in places the appearance of a reticulum, the mesh of which is in the neighborhood of a micron. Interspersed among the microsomes much larger granules are found here and there, connected by fine strands, which often consist of a row of microsomes, though in other cases the connecting strand appears as a homogeneous line. In other vacuoles (always of considerable size) the wall when seen in surface view exhibits coarse granules, set regularly and almost as closely as in the intervacuolar endosarc, between which run fine strands. If microsomes are present in such a wall, their presence is obscured by the coarser reticulum.

The ectosarc in even a thinly expanded pseudopodium looks nearly homogeneous ($\frac{1}{12}$ Zeiss) in the living animal. In acetic carmine preparations mounted in glycerine, and in alcohol or acetic methyl-green preparations mounted in water, the ectosarc may be seen to be occupied by a delicate reticulum, the mesh of which is close to 1 μ. I was not able to decide with certainty (working, however, only with Zeiss $\frac{1}{12}$) whether the microsomes are always situated at the points of intersection of the reticular lines (lamellæ of Bütschli), or whether each (apparently) linear side of a mesh included a short row of microsomes. Dispersed in this delicate reticulum, which seems identical with that seen in many vacuolar walls, are to be found here and there a few coarse granules.

The fine ectosarcal reticulum, in a fixed specimen examined in water under an unsupported cover, may be seen with a Zeiss $\frac{1}{12}$ to enter into the composition of the surface film. This now appears as a row of microsomes connected by strands. On the other hand, when the Pelomyxa is examined under a supported cover-glass with a dry lens, the superficial membrane has an appreciable thickness and is plainly doubly contoured. Blochmann[1] mentions that in his Pelomyxas the "Hautschicht" seems to have a double contour. The doubly contoured appearance of the surface membrane is, I believe, due to the arrangement of the superficial meshes (alveoli of Bütschli), which, as Bütschli[2] has shown in so many cases, are arranged in a layer with the partition walls vertical to the surface. That is, the membrane appears doubly contoured, because we see the outer and inner walls of the meshes of the superficial layer, and fail to distinguish the partition walls between the several meshes. Under slight pressure the reticular (alveolar) structure comes into view.

In these few notes on the finer structure of the cytoplasm I have used the terms "microsomes" and "reticulum" as indicating the optical appearances obtained, and not with the intention of expressing a belief that the structures as such have an actual existence.

In *P. palustris* the regularly arranged coarse granules, described above as present in vacuolar walls, are not figured by Gould (*loc. cit.*). In *P. carolinensis* they may easily be seen in the living specimen. The regularity of their arrangement at first sight suggests that they are not, to use some apt expressions of Mrs. Andrews,[3] mere "passive chemical inclusions," but are "physiological areas or substance organs," *i.e.*, permanently differentiated portions of the living substance. On the other hand, they are not always present throughout the extent of the vacuolar wall, and in many vacuoles are absent or nearly so. The presumption is thus a fair one that the bodies are mesostates, and owe their regular arrangement (in places) to the underlying fine reticulum.

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[1] Kleinere Mittheilungen über Protozoen, *Biol. Centralbl.*, Bd. xiv.

[2] *Investigations on Microscopic Foams and on Protoplasm.*

[3] *The Living Substance*, p. 16. Boston, 1897.

The number, shape, and size of the nuclei have already been alluded to. The chromatin granules are extremely numerous, especially abundant just within the nuclear membrane. They also form a densely crowded large mass in the center of the nucleus. Between the peripheral layer and central mass, the nucleoplasm comes into view more conspicuously than elsewhere. The granules are connected freely by intervening delicate strands, which themselves often show a row of finest granules. The granules, as a whole, are too crowded to permit decision as to whether a continuous reticulum exists. The chromatin granules vary in size from that of the cytoplasmic microsome up to much larger ones, 1 μ in diameter, the size of the common coarse granules in the wall of vacuoles.

Motion. — I regret not to have studied the currents of the body, when the animal is in those shapes in which it is only applied to the surface at isolated points (Figs. 3–7). At such times a locomotory motion, if it existed, escaped my notice. The animal was at rest for sufficiently long periods of time to permit careful study and drawing.

When the body is linear and applied throughout its extent to the surface, as in Figs. 1 and 2, the motion is, in general, similar to that of *P. villosa*,[1] or of the form commonly known as *Amœba limax.* As has already been said, *P. carolinensis*, when placed on the slide, assumes the habitus of *Amœba proteus* (Fig. 8). In this condition the movements are active, the pseudopodia being protruded in, or nearly in, a horizontal plane, but in any direction. The difference in appearance between pseudopodia into which the current is going, and those out of which it is flowing, is in this condition very noticeable. The contour of the former is smooth, the pseudopodia having an appearance of plumpness and distention. In the latter case, on the contrary, the surface is thrown into "mulberry-like" folds at the ends of the pseudopods. Moreover, it can frequently be seen in such a pseudopodium that the upper surface is thrown into longitudinal wrinkles. These are shown in two of the pseudopods in Fig. 8. The appearance of these pseudopodia is in perfect harmony with Bütschli's description (*loc. cit.*,

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[1] Leidy. *Fresh-Water Rhizopods of North America*, p. 73.

p. 313) of the backward flow of protoplasm from a pseudopodium : "The viscid external layer cannot flow together rapidly enough to keep pace with this diminution, in consequence of which it is thrown into folds, etc." Returning to the expanding pseudopodium, I may say that Wallich's remarks on *Amœba villosa,* quoted by Leidy (*loc. cit.*, p. 75) as applying to *P. villosa,* aptly describe the appearances to be had in *P. carolinensis.* After the local thickening of ectosarc, which precedes the formation of a pseudopodium, there is a *sudden* inrush of granules. The very suddenness of this inrush inevitably suggests that something in front has burst or given way, rather than that something behind has *begun* to contract, and, as far as it goes, is an argument for Bütschli's theory (*loc. cit.*, p. 311), that the formation of a pseudopodium is primarily due to the bursting of superficial alveoli, whereby (through the action of the enchylema on the free surface of the protoplasm) the surface tension in that locality is diminished.

Systematic. — In the assumption of complexly branched shapes (Figs. 6 and 7) *P. carolinensis* differs from the described conditions of the type species, *P. palustris* Greef, and of *P. villosa* Leidy, which are habitually more or less slug-like when in motion, and of massive shape when at rest. The peculiar posterior "villi" characteristic of the latter form are absent in *P. carolinensis,* which, moreover, differs from both forms in the fact that it does not gorge itself with mud, and that it contains no "Stäbchen" or symbiotic bacteria, as they are believed to be by Gould (*loc. cit.*) and some other observers. The Glanzkörper (albuminous globules) are present in Greef's species, and Leidy describes them in *P. villosa.* Blochmann (*loc. cit.*) and Frenzel (*loc. cit.*), however, mention their absence in the forms which they identified as belonging to *P. villosa.* The minute crystals which I find so abundantly in *P. carolinensis* are apparently not present in *P. palustris.* Neither Greef nor Gould mentions them, nor were they found by Leidy in *P. villosa.* Possibly the granules which Blochmann finds in such quantity in some of his Pelomyxas correspond to my "crystals." The Pelomyxas in which Blochmann finds the granules abundant have large and conspicuous Glanzkörper, round which the Stäbchen never form a mantle. Blochmann groups these forms under *P. palustris.* Other forms Blochmann unites under the provisional species, *P. greefi*, characterized by the almost complete absence of the granules, and by the small number of Glanzkörper, which are always surrounded by a thick coating of Stäbchen. The peculiar habitus assumed at times, absence of sand in the body, absence of Stäbchen, presence of the crystals, are the distinguishing characteristics of my species against the related forms, *P. palustris*, *P. villosa*, *P. greefi*.

The habitus in rhizopods, however, is undoubtedly a most inconstant feature, readily affected by the individual environment, as may be learned from *P. carolinensis* itself. The interesting observations of Verworn (*Allgemeine Physiologie*, p. 190) on the change of shape in amœbas, through the *A. proteus*, *A. limax*, and *A. radiosa* condition, likewise indicate this. And it seems probable, from the diverse forms of Pelomyxa, that Blochmann (*loc. cit.*) has found that the cytoplasmic inclusions, even the characteristic Glanzkörper, are all inconstant, and are to be looked on as the outcome of the individual environment. A greater familiarity with the four above-mentioned forms may thus show that they all belong to a single species.

UNIVERSITY OF NORTH CAROLINA,
December 18, 1899.